tive effects with nitrous oxide. While these experiments do not establish the mechanism for the teratogenicity of nitrous oxide, it is clearly not related to the intrinsic mechanisms of anesthesia, since xenon is as potent as nitrous oxide. Such factors as stress and the presence of additional atmospheric pollutants may also be excluded.

Why, then, is nitrous oxide teratogenic? First, it is chemically reactive and may undergo biotransformation (*9*) to metabolites that could be toxic; this cannot occur with xenon. Second, changes in uterine and fetal blood flow due to the cardiovascular effects of nitrous oxide could affect fetal development. Finally, nitrous oxide has peculiar effects on hematopoiesis. Prolonged administration of nitrous oxide to humans causes leucopenia and megaloblastic anemia (*10*). Nitrous oxide inactivates vitamin B_{12} by oxidizing cobalamin (*11*), inhibiting the conversion of homocysteine to methionine (*12*). This in turn affects thymidine (and perhaps DNA) synthesis. The clinical implications of the possibility that nitrous oxide may affect both hematopoiesis and organogenesis by inhibiting vitamin B_{12}-dependent reactions are especially challenging.

Should xenon replace nitrous oxide for clinical anesthesia? Calculations indicate that the atmosphere does not contain enough xenon for general clinical use with current techniques of anesthesia. As Cullen (*13*) said of xenon in 1951, "although it may not by virtue of its cost of manufacture prove to be a satisfactory agent commercially, it may materially assist in solving one of the important theoretical problems of anesthesia."

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Cellular Transplantation in the Treatment of Experimental Hepatic Failure

Abstract. *The survival of Lewis rats with D-galactosamine-induced fulminant hepatic failure was prolonged if they were given intraperitoneal injections of single-cell suspensions of liver or bone marrow cells from normal rats. Suspensions of liver cells were also effective in prolonging the survival of rats with ischemia-induced hepatic necrosis. The liver cells did not act by repopulating the recipient liver.*

There is no satisfactory treatment for fulminant hepatic failure in man (*1*, *2*). The variability of the disease and hence difficulties with controlled trials in humans makes it necessary to find a suitable animal model of the disease in which to assess potential therapy aimed at maintaining hepatic function until hepatic regeneration occurs. Here we describe the efficacy of hepatocellular and bone marrow cell transplantation in the reversal of acute hepatic necrosis induced in rats.

A consistent model of lethal hepatic necrosis was produced in 2- to 4-month old inbred male Lewis rats (*3*) weighing 280 to 330 g by the intraperitoneal injection of D-galactosamine hydrochloride (Sigma). When 50 animals were injected with D-galactosamine (2.6 g per kilogram

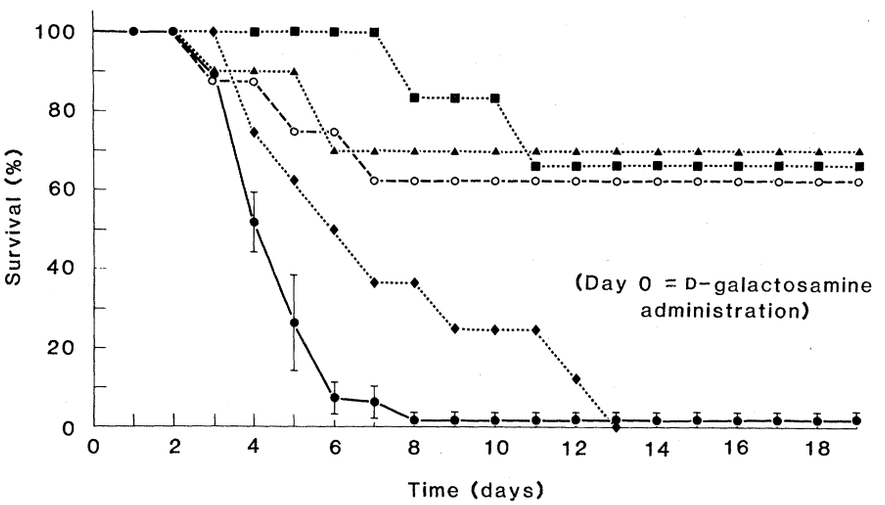

Fig. 1. Percentage of survival of D-galactosamine-injected Lewis rats after they received syngeneic liver cells. Single-cell suspensions of liver cells (4×10^7 cells per rat) were injected intraperitoneally at 24, 48, and 60 hours after toxin administration. Viability of the liver cells, as determined by trypan blue exclusion, was 85 ± 5 percent. Although hepatocytes given at 24 hours (◆) prolonged survival, all animals succumbed by day 13 ($N = 16$). When hepatocytes were given at 48 hours (▲) ($N = 16$) and at 60 hours (■) ($N = 12$), 70 and 66 percent of the rats survived, respectively ($P < .001$). By day 14 these animals showed normal liver function and structure. Also shown here is the influence of nonreplicating liver cells on survival after D-galactosamine administration. Single-cell suspensions of syngeneic liver cells received 10,000 rads from a cesium source and were then injected intraperitoneally (4×10^7 cells per rat; 50 percent viability by trypan blue exclusion) 48 hours after toxin administration. These nonreplicating cells increased survival to 62.5 percent (○) ($N = 16$, $P < .001$). The control curve (●) shown here represents the mean (± standard error) survival for three separate experiments ($N = 82$).

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of body weight), 94 percent died within 3 to 8 days. Maximum mortality occurred on days 4 and 5, with no deaths occurring during the first 48 hours, allowing adequate time for therapeutic intervention.

The effect of syngeneic liver cell suspensions prepared by a modification of Berry and Friend's technique (*4*) of perfusion ex vivo and collagenase digestion was assessed in this model of hepatic necrosis. The rats were injected intraperitoneally with suspensions of syngeneic cells at intervals after galactosamine administration. Although survival was prolonged to some extent in all instances, long-term survival was only brought about by transplantation of liver cells 40, 48, and 60 hours after toxin administration (Fig. 1).

Since liver cell suspensions prepared by the present method contain a proportion of Kupffer cells (*5*), the prolonged survival might have been due to this cell population and not hepatocytes. We therefore assessed the effect of other reticuloendothelial cells. Bone marrow cells were chosen initially because of ease of preparation and the high proportion of macrophages. The intraperitoneal injection of single-cell suspensions of bone marrow cells was successful in prolonging survival, with the optimal time of administration being 24 hours after the injection of D-galactosamine hydrochloride (Fig. 2). Single-cell suspensions of splenocytes and thymocytes teased through wire mesh, and of macrophages either induced by intraperitoneal administration of Bacillus Calmette-Guérin (Connaught Laboratories, Toronto) or purified by cell culture from bone marrow (*6*), were prepared and administered to D-galactosamine-injected rats at intervals. None of these treatments had any effect on survival.

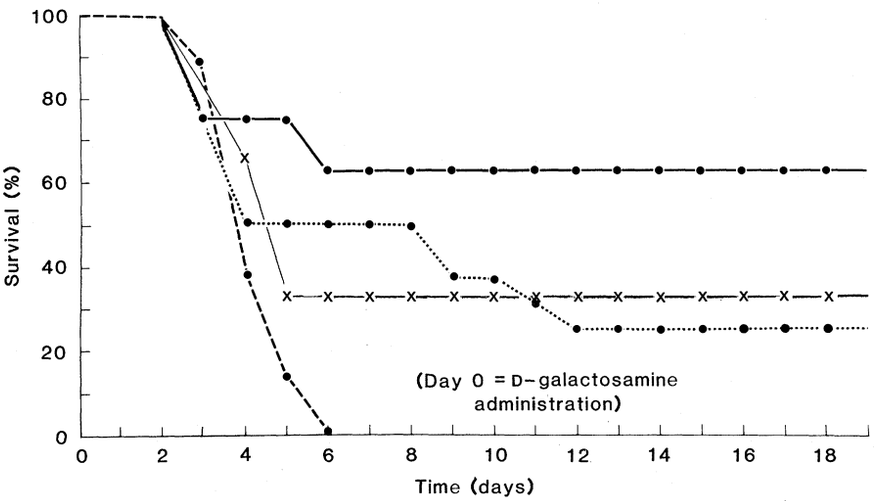

Fig. 2. The effect of transplantation of single-cell suspensions of bone marrow after D-galactosamine administration. Syngeneic bone marrow suspensions were prepared by washing the long bones of rats' hind limbs with normal saline at 4°C. The cells were injected intraperitoneally (4×10^7 cells per rat) at 24, 48, and 60 hours after toxin administration. Control animals (• - - •) ($N = 16$) were all dead by day 6. The survival of rats treated at 24 hours (• — •) was 62.5 percent ($N = 16, P < .001$). Survival of the rats treated at 48 hours (• ···· •) ($N = 16$) and 60 hours (X—X) ($N = 12$) did not differ significantly from control.

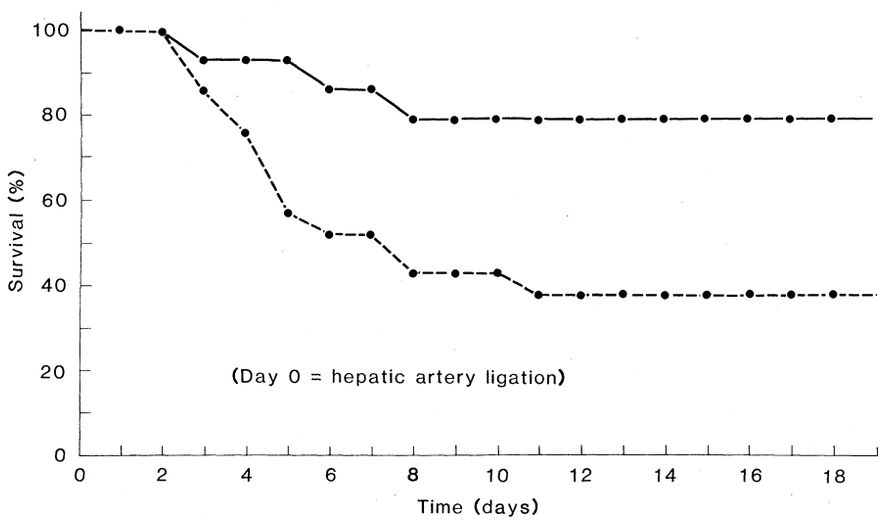

Fig. 3. The effect of liver cell transplantation on the survival of Lewis rats with anoxia-induced hepatic necrosis. The rats were anesthetized with ether and under aseptic conditions the hepatic artery was ligated distal to the gastroduodenal artery. The intraperitoneal administration of 4×10^7 syngeneic liver cells at 48 hours after ligation increased the number of rats surviving to 79 percent (• — •) ($N = 14$) compared to 38 percent for controls (• - - •) ($N = 21, P < .05$).

To determine whether the prolonged survival produced by liver cell and bone marrow transplantation was due to the metabolism and detoxification of any remaining galactosamine, we injected the nucleotide uridine (Sigma) (1.2 mg per gram of body weight) into rats that had received D-galactosamine 24 and 48 hours previously (*7*, *8*). The animals treated with uridine showed no improvement in their survival compared to controls, indicating that galactosamine detoxification at these times was not a factor in increasing survival.

The effect of hepatocellular transplantation in acute hepatic ischemia was studied to determine whether the improved survival was specific to galactosamine-induced hepatic failure. Ligation of the hepatic artery in Lewis rats produced 60 to 70 percent mortality. The administration of liver cells prepared as above and given 48 hours after hepatic artery ligation significantly improved survival in this model (Fig. 3). Therefore, liver cell transplantation could improve survival in two independent models of hepatic necrosis. We then determined if this effect was due to repopulation of the recipient liver by the transplanted cells. Suspensions of liver cells, prepared as above, were irradiated with 10,000 rads (cesium source; Atomic Energy Canada) and injected intraperitoneally into rats injected 48 hours previously with D-galactosamine. The irradiated cells retained their capacity to prolong the survival of the rats (Fig. 1).

In a further experiment, rats were subjected to hepatectomy in which 70 percent of the liver was removed. The remaining liver cells were then labeled in vivo by intraperitoneal injection of [^{3}H]thymidine (New England Nuclear) every 8 hours for 7 days after the hepatectomy. The livers from these animals were then perfused as above and the labeled single-cell suspensions thus obtained were used to treat animals with

galactosamine-induced hepatic necrosis. The surviving recipient animals were killed at intervals over 2 weeks. Liver sections were examined by radioautography for the presence of labeled cells. There was no significant uptake of the labeled donor cells by the regenerating livers. Therefore, the transplanted liver cells did not repopulate the recipient injured livers. Moreover, allogeneic (Buffalo and ACI strain) and xenogeneic (porcine) liver cells were as effective as syngeneic cells in prolonging survival after galactosamine administration (*9*). Unless the liver is a privileged site, the rapid destruction of these foreign cells by immune mechanisms argues further against repopulation.

We have shown that either liver or bone marrow cells can prolong the survival of rats with experimental acute hepatic failure. Liver cells can reverse the hepatic failure brought about by toxic or ischemic necrosis. The effect of bone marrow does not appear to be mediated by macrophages, but may be dependent on some other cell type yet to be isolated.

However, the recent evidence indicating that hepatic macrophages (Kupffer fer cells) are of bone marrow origin (*10, 11*) raises interesting questions concerning the role of cells derived from bone marrow. Repopulation of the liver by the transplanted cells does not occur. Whether these cells maintain metabolic support during the period of maximum hepatic failure, or whether a factor derived from these cells increases the rate of regeneration in the recipient liver remains to be determined.

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Calcofluor White ST Alters the in vivo Assembly of Cellulose Microfibrils

Abstract. *The fluorescent brightener, Calcofluor White ST, prevents the in vivo assembly of crystalline cellulose microfibrils and ribbons by* Acetobacter xylinum. *In the presence of more than 0.01 percent Calcofluor,* Acetobacter *continues to synthesize high-molecular-weight β-1,4 glucans. X-ray crystallography shows that the altered product exhibits no detectable crystallinity in the wet state, but upon drying it changes into crystalline cellulose I. Calcofluor alters cellulose crystallization by hydrogen bonding with glucan chains. Synthesis of this altered product is reversible and can be monitored with fluorescence and electron microscopy. Use of Calcofluor has made it possible to separate the processes of polymerization and crystallization leading to the biogenesis of cellulose microfibrils, and has suggested that crystallization occurs by a cell-directed, self-assembly process in* Acetobacter xylinum.

Calcofluor White ST (4,4′-bis[4-anilino-6-bis(2-hydroxyethyl)amino-*s*-triazin-2-ylamino]-2,2′-stilbenedisulfonic acid) is a fluorescent brightener used commercially to whiten cellulosic textiles and paper. Because of its ability to hydrogen bond with β-1,4 and β-1,3 polysaccharides (*1*) as a vital stain (*2*), it has also been used by biologists to localize cellulose and chitin (*3*). In this report we describe results indicating that Calcofluor White ST alters cellulose synthesis in the Gram-negative bacterium *Acetobacter xylinum* by separating the polymerization of high-molecular-weight β-1,4 glucans from their crystallization into cellulose I microfibrils.

Acetobacter xylinum normally produces crystalline 30-Å microfibrils in association with intracellular synthesizing sites and a longitudinal array of extrusion sites in the lipopolysaccharide layer of the bacterium (*4*). The microfibrils hydrogen bond together (*5*) into an extracellular ribbon of cellulose (Fig. 1, a and b); ribbons from many bacteria intertwine into a tough pellicle on the surface of the culture medium.

When actively synthesizing *A. xylinum* cells are incubated in medium or phosphate buffer containing more than 0.01 percent Calcofluor, ribbon assembly is disrupted (Fig. 1, e and f). Instead of twisting ribbons, the bacteria synthesize broad bands of bent fibrils. High-resolution micrographs reveal that the smallest fibrils in the band product measure 15 Å (±4 Å) (Fig. 1i). Larger fibrils appear to arise by fasciation of the smaller 15-Å fibrils. These 15-Å fibrils frequently show pronounced curvature and undulation, which suggests low crystallinity (*6*), while the larger aggregates often bend sharply and appear more rigid.

The altered cellulose synthesized is known to be high-molecular-weight, noncrystalline β-1,4 glucans by the following evidence: (i) the band is rapidly degraded by purified cellulase (*7*); (ii) radioactive glucose is incorporated into an alkali-insoluble product (*8*) and into a band next to the cell, as visualized by autoradiography in the presence of Calcofluor; (iii) viscosity measurements show that the Calcofluor-induced product has a degree of polymerization comparable to that of control samples (*9*); and (iv) x-ray crystallography studies indicate that the Calcofluor-induced product has cellulose I crystallinity after drying although it has no detectable crystallinity in the wet state (*10*). This lack of crystallinity before drying indicates that the Calcofluor-induced product is profoundly different from native cellulose of *A. xylinum*, which has about the same cellulose I crystallinity (*11*) in the wet and dry states, the crystallite size being 65 and 74 Å, respectively. The size of the cellulose I crystallites obtained after drying of the altered cellulose depends on the initial concentration of Calcofluor—69-Å crystallites resulting if a concentration of 0.025 percent is used and 28-Å crystallites if 0.1 percent or 0.5 percent concentration is used to induce the alteration.

The noncrystalline nature of the un-

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